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REPORT DOCUMENTATION PAGE

AD-A203 095

1b. RESTRICTIVE MARKINGS DTIC FILE COPY

3. DISTRIBUTION/AVAILABILITY OF REPORT
Approved for public release; distribution unlimited.

2b. DECLASSIFICATION/DOWNGRADING SCHEDULE

4. PERFORMING ORGANIZATION REPORT NUMBER(S)

5. MONITORING ORGANIZATION REPORT NUMBER(S)
ARO 22924.7-LS

6a. NAME OF PERFORMING ORGANIZATION
Stanford University

6b. OFFICE SYMBOL (If applicable)
N/A

7a. NAME OF MONITORING ORGANIZATION
U. S. Army Research Office

6c. ADDRESS (City, State, and ZIP Code)
Department of Chemistry
Stanford, CA 94305

7b. ADDRESS (City, State, and ZIP Code)
P. O. Box 12211
Research Triangle Park, NC 27709-2211

8a. NAME OF FUNDING/SPONSORING ORGANIZATION
U. S. Army Research Office

8b. OFFICE SYMBOL (If applicable)

9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
DAAG29-85-K-0242

8c. ADDRESS (City, State, and ZIP Code)
P. O. Box 12211
Research Triangle Park, NC 27709-2211

10. SOURCE OF FUNDING NUMBERS

PROGRAM ELEMENT NO.	PROJECT NO.	TASK NO.	WORK UNIT ACCESSION NO.

11. TITLE (Include Security Classification)
Organic Phosphonates: Disruption of Plant Metabolism & Degradation by Microorganisms

12. PERSONAL AUTHOR(S)
John W. Frost and Richard N. Zare

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Final	FROM 10/01/85 TO 09/30/88	12/12/88	6

16. SUPPLEMENTARY NOTATION
The view, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy, or decision, unless so designated by other documentation.

17. COSATI CODES		
FIELD	GROUP	SUB-GROUP

18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

Novel laser-based analytical methodology was developed to determine the fate of the phosphorus portion of organophosphonates immediately after cleavage of the carbon to phosphorus bond by Escherichia coli.

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT
☐ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS

21. ABSTRACT SECURITY CLASSIFICATION
Unclassified

22a. NAME OF RESPONSIBLE INDIVIDUAL

22b. TELEPHONE (Include Area Code)

22c. OFFICE SYMBOL

DD FORM 1473, 84 MAR 83 APR edition may be used until exhausted. All other editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE
UNCLASSIFIED

ARO 22924.7-LS

FINAL TECHNICAL REPORT
J.W. Frost/ R.N. Zare
DAAG29-85-K-0242

Our goal for the overall research project was to obtain insight into the mechanism used by microbes to cleave the carbon to phosphorus (C-P) bond of organophosphonates. Escherichia coli whole cells and cell lysate were first examined by ^{31}P NMR which did not reveal any degradation products. However, subsequent analysis of volatiles produced in sealed growths of E. coli cultured in medium where alkylphosphonic acids were the only source of phosphorus led to the discovery of hydrocarbons. (Cordeiro, M.L.; Pompliano, D.L.; Frost, J.W. J. Am. Chem. Soc. 1986, 108, 332). Analysis of the volatiles relied on gas chromatographic separation and flame ionization detection.

With the discovery of methane formation when E. coli were grown on medium having methylphosphonic acid as the only phosphorus source, all of our hypotheses were invalidated as to how mechanistically the microbes were cleaving the organophosphonate C-P bond. Our efforts were further complicated by the lack of C-P cleavage activity in cell-free lysate of E. coli. Circumvention of these impediments has exploited chemical modeling, identification of products and intermediate metabolites involved in the biodegradation, and molecular biological analysis.

Carbon fragments produced by E. coli degradation of a wide range of alkyl- and alkenylphosphonates have been compared with the carbon fragments produced by various chemical methods developed for cleaving organophosphonate C-P bonds. (Frost, J.W.; Loo, S.; Cordeiro, M.L.; Li, D. J. Am. Chem. Soc. 1987, 109, 2166). Correspondence of the products produced by the chemical degradation to those produced by the biotic degradation led to the proposal for a radical-based dephosphorylation process during organophosphonate C-P bond cleavage.

Two types of radical-based dephosphorylation processes can be envisioned. One formulation involves intermediacy of a phosphoranyl radical while the other proceeds through a phosphonyl radical. (AW) Differentiation between these mechanistic hypotheses requires knowledge as to the fate of the phosphorus portion of organophosphonates immediately before and after cleavage of the C-P bond. To accomplish this task, derivatization techniques suitable for laser-induced fluorescence detection were developed. Derivatization of alkylphosphonic acids with p-(9-anthroyloxy)phenacyl bromide provided nearly quantitative yields of dipanacyl organophosphonates. The detection limit for these derivatized organophosphonates was 20 fmol using 8 mW of 325-nm radiation from a He-Cd laser (Roach, M.C.; Ungar, L.W.; Zare, R.N.; Reimer, L.M.; Pompliano, D.L.; Frost, J.W. Anal. Chem. 1987, 59, 1056). Unfortunately, the derivatization reagent did not esterify phosphoric acid or phosphorous acid which are two possible phosphorus-containing degradation products. Resolution of this problem required increasing the reactivity of the derivatization reagent. We

have since discovered that a phenacyl group containing a triflate was reactive enough to esterify phosphoric acid, phosphorous acid, ethylphosphonic acid, and ethylphosphonous acid. Simultaneous with the laser-based detection system development, we have been optimizing methods for introduction of ^{32}P into organophosphonates. This latter methodology is intended for capillary zone electrophoretic separation of metabolites interfaced with β emission detection.

The final area of activity where significant progress has been made ~~involves~~ molecular biological characterization of microbial C-P bond cleavage. During anaylsis of aminomethylphosphonate biodegradation by E. coli, carbon fragments were generated which suggested a biodegradative mechanism similar to alkylphosphonate C-P bond cleavage. E. coli were then subjected to transposon mutagenesis. Mutants unable to degrade ethylphosphonate were appraised for ability to degrade aminomethylphosphonates. One mutant, E. coli SL724, was unable to degrade both alkyl- and aminomethylphosphonates. This mutant thus establishes a linkage at the genetic level between biodegradation of these two very different classes of organophosphonates (Avila, L.Z.; Loo, S.H.; Frost, J.W. J. Am. Chem. Soc. 1987, 109, 6758). The gene which complements the mutation of SL724 has recently been cloned and used to establish the genomic location of the locus responsible for organophosphonate C-P bond cleavage (Loo, S.H.; Peters, N.K.; Frost, J.W. Biochem. Biophys. Res. Commun. 1987, 148, 148).

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22924-LS

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